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PERKINS COIE LLP P.O. BOX 2168 MENLO PARK, CA 94026			BARTON, JEFFREY THOMAS	
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Please find below and/or attached an Office communication concerning this application or proceeding.

5

Interview Summary	Application No. 10/034,278	Applicant(s) KURNIK ET AL.	
	Examiner Jeffrey T. Barton	Art Unit 1753	

All participants (applicant, applicant's representative, PTO personnel):

(1) Jeffrey T. Barton. (3) _____.

(2) Lee Ann Gorthey. (4) _____.

Date of Interview: 02 February 2006.

Type: a) ☒ Telephonic b) ☐ Video Conference
c) ☐ Personal [copy given to: 1) ☐ applicant 2) ☐ applicant's representative]

Exhibit shown or demonstration conducted: d) ☒ Yes e) ☐ No.

If Yes, brief description: Verheggen et al., J. Chromatogr. 452, 615-622 (1988).

Claim(s) discussed: _____.

Identification of prior art discussed: Manz et al, Krivankova et al, Verheggen et al.

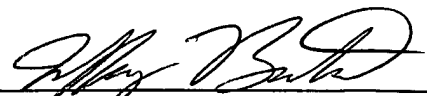
Agreement with respect to the claims f) ☐ was reached. g) ☒ was not reached. h) ☐ N/A.

Substance of Interview including description of the general nature of what was agreed to if an agreement was reached, or any other comments: See Continuation Sheet.

(A fuller description, if necessary, and a copy of the amendments which the examiner agreed would render the claims allowable, if available, must be attached. Also, where no copy of the amendments that would render the claims allowable is available, a summary thereof must be attached.)

THE FORMAL WRITTEN REPLY TO THE LAST OFFICE ACTION MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW. (See MPEP Section 713.04). If a reply to the last Office action has already been filed, APPLICANT IS GIVEN ONE MONTH FROM THIS INTERVIEW DATE, OR THE MAILING DATE OF THIS INTERVIEW SUMMARY FORM, WHICHEVER IS LATER, TO FILE A STATEMENT OF THE SUBSTANCE OF THE INTERVIEW. See Summary of Record of Interview requirements on reverse side or on attached sheet.

Examiner Note: You must sign this form unless it is an Attachment to a signed Office action.


Examiner's signature, if required

Summary of Record of Interview Requirements

Manual of Patent Examining Procedure (MPEP), Section 713.04, Substance of Interview Must be Made of Record

A complete written statement as to the substance of any face-to-face, video conference, or telephone interview with regard to an application must be made of record in the application whether or not an agreement with the examiner was reached at the interview.

Title 37 Code of Federal Regulations (CFR) § 1.133 Interviews

Paragraph (b)

In every instance where reconsideration is requested in view of an interview with an examiner, a complete written statement of the reasons presented at the interview as warranting favorable action must be filed by the applicant. An interview does not remove the necessity for reply to Office action as specified in §§ 1.111, 1.135. (35 U.S.C. 132)

37 CFR §1.2 Business to be transacted in writing.

All business with the Patent or Trademark Office should be transacted in writing. The personal attendance of applicants or their attorneys or agents at the Patent and Trademark Office is unnecessary. The action of the Patent and Trademark Office will be based exclusively on the written record in the Office. No attention will be paid to any alleged oral promise, stipulation, or understanding in relation to which there is disagreement or doubt.

The action of the Patent and Trademark Office cannot be based exclusively on the written record in the Office if that record is itself incomplete through the failure to record the substance of interviews.

It is the responsibility of the applicant or the attorney or agent to make the substance of an interview of record in the application file, unless the examiner indicates he or she will do so. It is the examiner's responsibility to see that such a record is made and to correct material inaccuracies which bear directly on the question of patentability.

Examiners must complete an Interview Summary Form for each interview held where a matter of substance has been discussed during the interview by checking the appropriate boxes and filling in the blanks. Discussions regarding only procedural matters, directed solely to restriction requirements for which interview recordation is otherwise provided for in Section 812.01 of the Manual of Patent Examining Procedure, or pointing out typographical errors or unreadable script in Office actions or the like, are excluded from the interview recordation procedures below. Where the substance of an interview is completely recorded in an Examiners Amendment, no separate Interview Summary Record is required.

The Interview Summary Form shall be given an appropriate Paper No., placed in the right hand portion of the file, and listed on the "Contents" section of the file wrapper. In a personal interview, a duplicate of the Form is given to the applicant (or attorney or agent) at the conclusion of the interview. In the case of a telephone or video-conference interview, the copy is mailed to the applicant's correspondence address either with or prior to the next official communication. If additional correspondence from the examiner is not likely before an allowance or if other circumstances dictate, the Form should be mailed promptly after the interview rather than with the next official communication.

The Form provides for recordation of the following information:

- Application Number (Series Code and Serial Number)
- Name of applicant
- Name of examiner
- Date of interview
- Type of interview (telephonic, video-conference, or personal)
- Name of participant(s) (applicant, attorney or agent, examiner, other PTO personnel, etc.)
- An indication whether or not an exhibit was shown or a demonstration conducted
- An identification of the specific prior art discussed
- An indication whether an agreement was reached and if so, a description of the general nature of the agreement (may be by attachment of a copy of amendments or claims agreed as being allowable). Note: Agreement as to allowability is tentative and does not restrict further action by the examiner to the contrary.
- The signature of the examiner who conducted the interview (if Form is not an attachment to a signed Office action)

It is desirable that the examiner orally remind the applicant of his or her obligation to record the substance of the interview of each case. It should be noted, however, that the Interview Summary Form will not normally be considered a complete and proper recordation of the interview unless it includes, or is supplemented by the applicant or the examiner to include, all of the applicable items required below concerning the substance of the interview.

A complete and proper recordation of the substance of any interview should include at least the following applicable items:

- 1) A brief description of the nature of any exhibit shown or any demonstration conducted,
- 2) an identification of the claims discussed,
- 3) an identification of the specific prior art discussed,
- 4) an identification of the principal proposed amendments of a substantive nature discussed, unless these are already described on the Interview Summary Form completed by the Examiner,
- 5) a brief identification of the general thrust of the principal arguments presented to the examiner,
(The identification of arguments need not be lengthy or elaborate. A verbatim or highly detailed description of the arguments is not required. The identification of the arguments is sufficient if the general nature or thrust of the principal arguments made to the examiner can be understood in the context of the application file. Of course, the applicant may desire to emphasize and fully describe those arguments which he or she feels were or might be persuasive to the examiner.)
- 6) a general indication of any other pertinent matters discussed, and
- 7) if appropriate, the general results or outcome of the interview unless already described in the Interview Summary Form completed by the examiner.

Examiners are expected to carefully review the applicant's record of the substance of an interview. If the record is not complete and accurate, the examiner will give the applicant an extendable one month time period to correct the record.

Examiner to Check for Accuracy

If the claims are allowable for other reasons of record, the examiner should send a letter setting forth the examiner's version of the statement attributed to him or her. If the record is complete and accurate, the examiner should place the indication, "Interview Record OK" on the paper recording the substance of the interview along with the date and the examiner's initials.

Continuation of Substance of Interview including description of the general nature of what was agreed to if an agreement was reached, or any other comments: Dr. Gorthey presented arguments that the cited portion of Manz et al does not suggest sample stacking, as stated in the motivation for the 103 rejection. She also pointed to unexpected benefits obtained through the method, which she indicated as being shown in Figure 6 of the disclosure. The Examiner agreed to consider these arguments. Since the case is currently under Final Rejection, both parties agreed that appropriate response should be sent by Applicant to continue prosecution.

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SIMPLE SAMPLING DEVICE FOR CAPILLARY ISOTACHOPHORESIS AND CAPILLARY ZONE ELECTROPHORESIS

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SUMMARY

A simple sampling device for isotachophoresis and zone electrophoresis is described, whereby the sample solution is introduced directly into a broadened part of the capillary tube by two feeders, placed perpendicularly to the capillary tube. The advantage of this sampling device is that with the absence of moving parts, automation of the sample introduction can be carried out in a simple way, cleaning is simple and the device is inexpensive. Experiments showed that a reproducibility of less than 2% can be obtained, including the day to day variations using different sample solutions.

INTRODUCTION

The sampling methods usually applied in capillary isotachophoresis (ITP) and capillary zone electrophoresis (CZE) are: injection with a syringe, via a septum, in an injection block, injection valves, with/without a sample loop, and dipping one end of the capillary tube into the sample solution, whereby the sample is introduced by (a) the gravity flow or (b) electroendosmosis and/or electromigration.

The best way of sampling in CZE is to introduce a representative aliquot of a sample, concentrated as much as possible in a narrow band and without mixing with the background electrolyte¹. However, none of the methods mentioned above was appropriate for this purpose. The use of a syringe can cause reading errors, especially for small amounts of sample, and a great disadvantage is the mixing of the sample with the background ionic species so that the sample cannot be introduced in a narrow band. In ITP this is of no importance because of the self-correcting effect of the zone boundaries.

A sample valve is¹, in fact, the most suitable sampling method for CZE. With a sample valve a known volume of a representative aliquot of the sample can be introduced without mixing with the background electrolyte. Using a sample valve in CZE it was very difficult, however, to avoid impurities coming from the "dead volumes" (*i.e.*, impurities coming from the liquid films) between moving parts because in CZE very small amounts of ionic species can be detected.

When a gravity flow², electroendosmosis or electromigration^{3,4} is applied an

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unknown volume is introduced so that an internal standard has to be used for quantitative analyses. In electroendosmosis and electromigration, moreover, the sample introduced often does not have the same composition as the original sample (see Discussion). Applying pulse injections with splitting techniques⁵ can be considered as electromigration with the advantage that a representative aliquot of the sample is taken.

In this paper a simple sampling device for capillary ITP and CZE is described, whereby the sample solution is introduced directly into part of the capillary tube by means of two feeders, perpendicular to the capillary tube. The sample can be introduced without mixing with the background electrolyte. With the absence of moving parts cleaning is simple. An advantage, as obtained with the use of a valve, is the concentration effect of dilute sample ionic species⁶.

EXPERIMENTAL

In order to examine the utility of this new sampling device, an apparatus was constructed as shown in Fig. 1. A cast capillary block C is connected between the electrode compartment A₁ and the sampling device SD. In the electrode compartments A₁ and A₂, the electrolyte solutions contact the Pt-Ir electrodes E. The capillary tube (0.25 mm I.D., separation length 6 cm) contains measuring electrodes M connected with a.c. electronics⁷. The sampling device SD consists of a broadened part of the capillary tube (0.55 mm I.D.) connected with two feeders (0.4 mm diameter), perpendicular to the capillary tube.

The electrolyte solutions are introduced using valves 1 and 3 and the drain 2. Valves 5 and 6 are used for complete rinsing of the electrode compartments. The sample can be introduced via valve 4 and the drain 2.

In first instance, the experiments were carried out with a closed valve, connected to drain 2. All data presented in this paper, however, were measured with drain 2 open, because identical results were obtained.

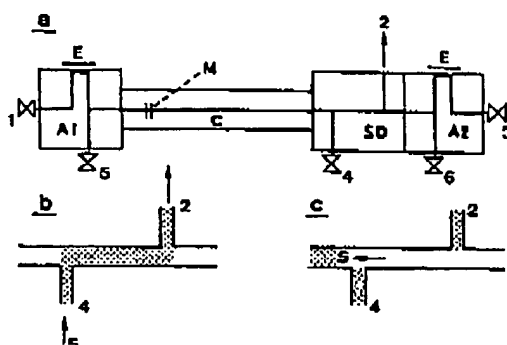


Fig. 1. (a) Schematic diagram of electrophoretic equipment with sample device (for further explanation, see text). (b) Sample device during sampling (S = sample). (c) Sample device after a certain time (S = sample).

SAMPLING DEVICE FOR CAPILLARY ITP

617

RESULTS

In order to study the feasibility, accuracy and reproducibility of the new sampling method, two questions have to be answered. The first is whether the sample device (SD) has a constant volume independent of the mobilities and concentrations of the sample components and the exact value of this SD volume. It is possible that, although no electric field gradient over the feeders exists, sample components from the feeders may diffuse into the capillary tube when the sample has already left the sampling position. This means that these sample ions, in the ITP mode, will be found in the terminating zone (with a very high electric field gradient) by means of which some of the sample ionic species can reach their own sample zones. This effect will lead to a greater zone length for high ionic mobilities and high concentrations.

The second question is whether reproducible and linear calibration graphs can be obtained for each sample component in both the ITP and CZE modes.

Determination of the volume of the sample device

For the determination of the SD volume, the fact has been used that equal amounts of ionic species always need equal times to pass a detector, whatever capillary diameters may be used in ITP analyses, provided that an equal electric current is applied.

Because in ITP the equation

$$E = j\sigma \quad (1)$$

holds, where j is the electric current density in A/cm², σ is the specific resistance in Ω cm and E is the electric field gradient in V/cm, the concentration of a specific sample zone is always constant if the same leading electrolyte is used. If, for example, the cross-sectional area of the capillary tube is increased, then the zone length decreases in proportion. Because the electric current density j decreases in a similar way, the zone velocity decreases, hence the time needed for the zone to pass the detector remains the same. This can also be formulated in another way, as follows. In order that a specific amount of a sample can pass a detector, a specific quantity of charge Q has to be transported. The quantity of transported charge will always be:

$$Q = It \quad (2)$$

Working at a constant electric current I a specific quantity of charge Q will pass in an equal time t . This fact was used to determine the volume of the sampling device.

Several different volumes of a sample solution were introduced into an ITP apparatus equipped with a capillary tube of 0.2 mm I.D. and an injection block. The sample volumes were introduced with a syringe and the volumes were read off under a microscope. Using the corresponding zone lengths a calibration graph was constructed.

The same sample solution was then introduced by the SD into the apparatus described in Fig. 1, and an ITP experiment was carried out using the same leading electrolyte and the same electric current. From the measured zone length the SD

618

Th. P. E. M. VERHEGGEN, J. L. BECKERS, F. M. EVERAERTS

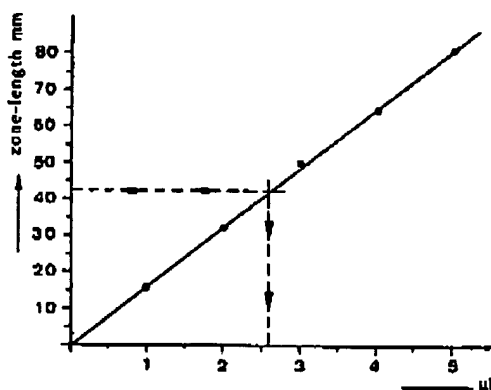


Fig. 2. Calibration graph for glutamate (0.001 *M*) in a leading electrolyte system at pH 6 (0.01 *M* hydrochloric acid + and 0.02 *M* histidine). The sample was introduced with a syringe and an injection block. From the zone length obtained using the sample device (SD) its volume can be obtained (dotted line).

volume can be obtained using the calibration graph for the ITP apparatus with injection block (see Fig. 2). This was carried out for different electrolyte systems and different sample solutions and the values obtained are given in Table I. It can be concluded from Table I that for ionic species with high ionic mobilities, a larger sample volume is found using the SD.

In order to establish whether the SD volume is also dependent on the concentration of the sample ionic species, the experiments were repeated with both apparatus for glutamate at different concentrations. The results are given in Table II, from which it can be concluded that the SD volume also depends on the concentration of the sample ionic species.

TABLE I

ZONE LENGTHS MEASURED WITH ITP EQUIPMENT WITH AN INJECTION BLOCK AND WITH THE SD FOR IONIC SPECIES AT A CONCENTRATION OF 0.001 *M*, AND CALCULATED SD VOLUMES

The leading electrolytes were 0.01 *M* hydrochloric acid + 0.02 *M* histidine at pH 6 and 0.01 *M* hydrochloric acid + 0.02 *M* ϵ -aminocaproic acid at pH 4.4. The terminator was MES. From the calibration graphs obtained with the injection block data and the zone length of the SD, the SD volume was calculated. The electric current was 25 μ A.

Ion	pH	Zone length using injection block (mm)					Zone length using SD (mm)	SD volume (μ l)
		1 μ l*	2 μ l*	3 μ l*	4 μ l*	5 μ l*		
Nitrate	6	13.7	24.4	36.7	48.3	59.1	34.1	2.80
Propionate		13.0	26.1	38.9	52.2	64.3	35.2	2.71
Glutamate		15.3	32.4	48.5	65.5	80.6	42.0	2.60
Nitrate	4.4	13.5	24.9	36.0	49.1	60.4	34.3	2.82
Propionate		12.7	25.6	37.4	52.0	64.3	33.5	2.61
Glutamate		14.8	31.6	46.7	62.7	77.3	39.5	2.54

* Injection volume.

SAMPLING DEVICE FOR CAPILLARY ITP

619

TABLE II

AS TABLE I FOR GLUTAMATE SOLUTIONS OF DIFFERENT CONCENTRATIONS

Concentration (M)	Zone length using injection block (mm)					Zone length using SD (mm)	SD volume (μ l)
	1 μ l*	2 μ l*	3 μ l*	4 μ l*	5 μ l*		
0.005	79.5	162.5	244.3	323.0	416.3	206.1	2.54
0.001	16.1	32.6	50.1	64.8	81.4	42.5	2.60
0.0005	8.1	16.1	24.4	33.5	40.2	21.6	2.65

* Injection volume.

The diffusion of sample ionic species from the feeders into the capillary tube cannot explain these two effects. The results in Table I, viz., larger effective SD volumes for ionic species with high mobilities, agree with the possibility of diffusion from the feeders, but the results in Table II, viz., higher effective SD volumes for smaller sample concentrations, do not. The explanation for the latter may possibly be that introducing low concentrations of sample ionic species means that the terminator solution at the original position of sampling will also have a low concentration according to Kohlrausch's law⁶. Hence high electric field strengths and high temperatures will result, which may be responsible for the larger effective SD volumes. The differences in SD volumes are, however, only a few percent.

Calibration

In order to check the reproducibility and linearity of the SD we measured the zone lengths for glutamate and acetate in an electrolyte system at pH 6 for several concentrations. The different concentrations were prepared by dilution of two different prepared standard solutions. The results are given in Table III, from which it can be concluded that linear calibration graphs were obtained with good reproducibility for the ionic species (see Table IV).

In order to check whether the calibration graphs are affected by the presence of other ionic species in the sample, we prepared several mixtures of acetate and glutamate and used the measured zone lengths to determine their concentrations in the sample mixture using the calibration graphs. In all instances the differences were less than about 2%.

To compare the results obtained with an injection block and the new SD, in Table IV all data for the calibration graphs are given. The results of a regression analysis are given, viz., the regression coefficient and the constants a and b according to the equation

$$Y = aX + b \quad (3)$$

where Y is the zone length (mm) and X is the volume injected (μ l) or the concentration (M).

From Table IV it can be concluded that the regression coefficients for the new sample device are better than those for the injection block. Although we used the average value of the zone length from five experiments for the new SD, it must be

620

Th. P. E. M. VERHEGGEN, J. L. BECKERS, F. M. EVERAERTS

TABLE III

ZONE LENGTHS FOR SEVERAL CONCENTRATIONS OF ACETATE AND GLUTAMATE MEASURED USING THE SD

All zone lengths were measured five times on different days. The leading electrolyte was 0.01 *M* hydrochloric acid + 0.02 *M* histidine at pH 6. The terminator was MES. The electric current was 25 μ A.

Ion	Concentration (M)	Zone length (mm)					Average zone length (mm)	Standard deviation (mm)
Acetate	0.0005	15.3,	15.4,	15.6,	15.7,	15.3	15.46	0.162
	0.001	30.5,	31.2,	30.1,	30.8,	31.1	30.74	0.403
	0.0015	44.9,	45.5,	44.7,	44.8,	44.6	44.9	0.316
	0.002	61.3,	60.3,	60.4,	59.3,	60.3	60.32	0.634
	0.0025	73.5,	74.3,	73.0,	73.0,	73.0	73.36	0.508
	0.005	144.3,	146.4,	143.6,	146.5,	144.3	145.02	1.196
	0.005	144.4,	144.4,	144.4,	144.0,	141.0	143.64	1.329
	0.010	294.8,	298.1,	297.4,	296.6,	296.9	297.76	1.104
Glutamate	0.0005	21.6,	21.4,	21.5,	21.9,	21.9	21.66	0.206
	0.001	42.3,	42.1,	42.1,	42.9,	42.2	42.32	0.299
	0.001	42.1,	42.4,	42.1,	42.1,	42.1	42.16	0.120
	0.002	83.6,	83.5,	83.0,	83.2,	83.5	83.36	0.224
	0.0025	103.4,	105.0,	104.6,	104.7,	105.0	104.54	0.592
	0.005	206.9,	209.9,	202.6,	205.2,	204.0	205.72	2.523
	0.005	202.7,	203.0,	202.7,	202.7,	205.3	203.28	1.017
	0.010	411.1,	407.1,	411.4,	407.4,	410.6	409.52	1.873

TABLE IV

CORRELATION COEFFICIENT AND THE CONSTANTS *a* AND *b* FOR THE REGRESSION ANALYSIS OF ALL CALIBRATION GRAPHS

Apparatus	pH	Ion	Constant <i>a</i> (mm/ μ l)	Constant <i>b</i> (mm)	Correlation coefficient
Injection block*	6	Nitrate	11.47	2.03	0.99950
		Propionate	12.87	0.29	0.99980
		Glutamate	16.19	0.0	0.99945
	4.4	Nitrate	12.18	0.0	0.99805
		Propionate	12.83	0.0	0.99899
		Glutamate	15.55	0.0	0.99948
Injection block**	6	Glutamate (0.005 <i>M</i>)	83.41	-5.11	0.99925
		Glutamate (0.001 <i>M</i>)	16.28	0.16	0.99940
		Glutamate (0.0005 <i>M</i>)	8.16	-0.02	0.99827
SD***	6	Acetate	29409.6 [§]	0.18	0.99954
		Glutamate	40752.2 [§]	1.53	0.99995

* Results in Table I.

** Results in Table II.

*** Results in Table III.

[§] mm · mol⁻¹ · l.

SAMPLING DEVICE FOR CAPILLARY ITP

621

borne in mind that all these experiments were carried out with different electrolyte solutions and on different days so that these deviations include all possible errors such as those coming from the sample device, day to day variations, the use of different stock solutions and the non-linearity of the calibration graphs.

From the foregoing experiments it can be concluded that the new SD has an effective volume that is dependent on the effective mobilities and concentrations of the sample ionic species. Calibration graphs, for the different ionic species, however, show a linear relationship between zone length and concentration of ionic species, independent on their mobilities and unaffected by the presence of other sample ionic species.

The dimensions of the feeders and the sampling channel were chosen arbitrarily. The choice of the optimal dimensions of the SD is under investigation, especially for the use of narrow-bore capillaries (50 μm).

In order to study the possibilities of automation, some experiments were carried out in which all electrolyte solutions and the sample were introduced by aspiration, using a simple peristaltic pump connected to drain 2. The results were identical with those obtained in the manual experiments, which means that the SD can be used for the automation of analyses.

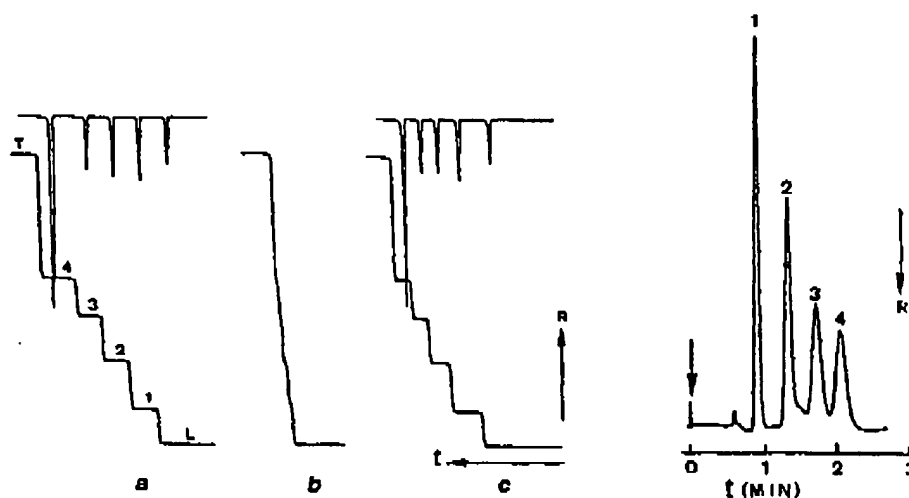


Fig. 3a. Isotachopherogram for the separation of (1) formate, (2) acetate, (3) benzoate and (4) glutamate. The leading electrolyte L was 0.01 *M* hydrochloric acid + and 0.02 *M* histidine at pH 6. The concentrations of the sample ionic species were $5 \cdot 10^{-4}$ *M*. The terminator T was 2-(N-morpholino)ethanesulphonic acid (MES). The electric current was 25 μA . (b) As (a) with sample concentrations of $5 \cdot 10^{-5}$ *M*. (c) As (b) with electromigration sampling.

Fig. 4. Electropherogram for the zone electrophoretic separation of (1) formate, (2) acetate, (3) benzoate and (4) glutamate. The concentrations of the sample ionic species were $2.5 \cdot 10^{-5}$ *M*. The background electrolyte was 0.01 *M* MES + 0.02 *M* histidine at a pH 6.4. The electric current was 25 μA .

DISCUSSION

Using the new sampling device a known amount of sample ionic species can be introduced and it has advantages over other sampling techniques in which a known sample is introduced. Compared with injection by a syringe this method has the advantage that there is no mixing with other electrolyte solutions, in either the ITP or the CZE mode. Compared with sampling valves the advantage is the absence of rotating parts. Compared with methods applying electromigration it has the advantage of introducing a representative aliquot of the sample (trapping⁹ or splitting⁵ have a similar advantage). To demonstrate the effect of applying electromigration for sampling three ITP experiments were carried out with the equipment described in Fig. 1, from which the isotachopherograms shown in Fig. 3 were obtained. Fig. 3a shows the separation of formate, acetate, benzoate and glutamate anions in a system of pH 6 and with concentrations of the sample ionic species of $5 \cdot 10^{-4}$ M. The separation was repeated with the same anions at concentrations of $5 \cdot 10^{-5}$ M (Fig. 3b) and the zone lengths are scarcely visible. In a third experiment (Fig. 3c) the sampling was carried out by electromigration, a dilute sample of $5 \cdot 10^{-5}$ M flowing through the SD for 30 s with application of an electric current. Although large zone lengths could be obtained, it can clearly be seen that no representative sample aliquot was introduced. The sample introduction follows a moving boundary procedure whereby larger amounts of ionic species with high mobilities are introduced.

With the same sample solution of concentrations $2.5 \cdot 10^{-5}$ M a zone electrophoretic experiment was also carried out and the electropherogram is shown in Fig. 4. The peaks are clearly visible, showing that very dilute samples easily can be handled using CZE. Further, the SD gives much greater possibilities of automating the sampling procedure both for ITP and CZE.

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